

UCSF

UC San Francisco Previously Published Works

Title

Selective proteolysis of immunoglobulins by mouse macrophage elastase.

Permalink

<https://escholarship.org/uc/item/1803b2v7>

Journal

The Journal of experimental medicine, 157(4)

ISSN

0022-1007

Authors

Banda, MJ
Clark, EJ
Werb, Z

Publication Date

1983-04-01

DOI

10.1084/jem.157.4.1184

Peer reviewed

SELECTIVE PROTEOLYSIS OF IMMUNOGLOBULINS BY MOUSE MACROPHAGE ELASTASE*

BY MICHAEL J. BANDA,‡ ELIZABETH J. CLARK, AND ZENA WERB

*From the Laboratory of Radiobiology and Environmental Health, University of California,
San Francisco, California 94143*

Macrophages interact with immunoglobulin G via cell surface receptors that recognize and bind the Fc portion of IgG. In the mouse, these Fc receptors (FcR)¹ are specific for IgG subclasses. A common FcR that is resistant to trypsin recognizes aggregated or immune-complexed IgG₁ and IgG_{2b} at high affinity but has low affinity for the monomeric proteins (1-3). There is a distinct FcR with similar properties for IgG₃ (4). A third FcR is trypsin sensitive and has high affinity for monomeric IgG_{2a} (1, 2). Cellular functions can be regulated by both the intact IgG molecule and by proteolytic fragments of IgG. Although not all the functions of Fc-mediated binding of intact IgG or IgG in immune complexes to the cell surface FcR are clearly understood, the IgG-FcR interaction mediates endocytosis of immune complexes and antibody-dependent cell-mediated cytotoxicity (5).

Soluble Fc fragments from degradation of IgG by papain have also been shown to potentiate the immune response (6). These fragments can increase endogenous production of prostaglandin E by monocytes and of IgM by peripheral blood cells (7). Fc fragments and subfragments have also been shown to be lymphocyte activators (8-11).

Because binding to the FcR involves the structure of the whole IgG molecule (12), proteinases that degrade the IgG molecule are likely to interfere with the function of the FcR and act as potential immunoregulators. Cells found at the site of acute and chronic inflammation, such as inflammatory macrophages, may be a source of IgG-degrading proteinases. One of the proteinases secreted by inflammatory macrophages is elastase (13). This metalloproteinase is a neutral endopeptidase capable of degrading elastin (14) as well as other proteins such as α_1 -proteinase inhibitor (15). In the study reported here, we investigated the proteolytic activity of mouse macrophage elastase on specific subclasses of mouse monoclonal IgG.

Materials and Methods

Materials. Dulbecco's modified Eagle's medium (complete and methionine free) was obtained from the Tissue Culture Facility, University of California, San Francisco, CA. Tissue-culture flasks (T75; Costar, Data Packaging, Cambridge, MA) were purchased from Microbiological Associates (Walkersville, MD). Penicillin, streptomycin, fetal bovine serum, and Dulbecco's phosphate-buffered saline were purchased from Grand Island Biological Co. (Grand

* Supported by the U. S. Department of Energy.

‡ Supported by grant HL26323 from the National Institutes of Health.

¹ Abbreviations used in this paper: Ac(Ala)₄CH₂Cl, acetyl-Ala-Ala-Ala-Ala-chloromethyl ketone; FcR, Fc receptor; PMSF, phenylmethanesulfonylfluoride; SDS, sodium dodecyl sulfate; SRBC, sheep erythrocytes.

Island, NY). IgG₁ (MOPC 21), IgG_{2a} (UPC 10, RPC 5), IgG_{2b} (MOPC 195, MOPC 141), IgG₃ (FLOPC 21, Y 5606), IgM (TEPC 183), and IgA (MOPC 315) were obtained as purified mouse myeloma proteins from Bionetics Laboratory Products (Kensington, MD). Monoclonal mouse anti-sheep erythrocyte (SRBC) IgG₃ was purified from culture medium of N-S.7 cells (16), and IgG_{2b} anti-Ia^k was purified from culture medium of 10-2.16 cells (Cell Distribution Center, Salk Institute for Biological Studies, San Diego, CA). Monoclonal mouse IgG_{2b} (Sp2/HL) and IgG₁ (Sp3/HL) were purified from culture medium obtained from Accurate Chemical & Scientific Corp. (Westbury, NY). Human IgG₁ was a gift of G. Crabtree of the National Institutes of Health, Bethesda, MD. Human α_1 -proteinase inhibitor (α_1 -antitrypsin) was purchased from Worthington Biochemical Corp. (Freehold, NJ). Tris (Trizma base), sodium dodecyl sulfate (SDS), 1,10-phenanthroline, phenylmethanesulfonylfluoride (PMSF), EDTA, and 2-mercaptoethanol were purchased from Sigma Chemical Co. (St. Louis, MO). All electrophoresis reagents (electrophoresis grade) were purchased from Bio-Rad Laboratories (Richmond, CA). [³⁵S]Methionine (>1,200 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL). Protein A-Sepharose was purchased from Pharmacia Fine Chemicals (Piscataway, NJ). IgSorb was purchased from the Enzyme Center (Boston, MA). Acetyl-Ala-Ala-Ala-Ala-chloromethyl ketone [Ac(Ala)₄CH₂Cl] was a gift of J. C. Powers, Georgia Institute of Technology, Atlanta, GA. YM10 and XM50 ultrafiltration membranes were purchased from Amicon Corp. (Danvers, MA).

Macrophage Elastase. The active form of macrophage elastase was purified from medium conditioned by thioglycollate-elicited mouse peritoneal macrophages as previously described (14). The preparations used were >5,000 U/mg protein.

Polyacrylamide Gel Electrophoresis. SDS-polyacrylamide gels were prepared and run by a modification of the method of Laemmli (17). Samples were applied to a 3% (wt/vol) polyacrylamide stacking gel on a linear 8–20% (wt/vol) polyacrylamide gel slab (0.75 × 10 × 10 cm). Gels were run at room temperature at 20 mA/gel. After electrophoresis the protein bands were fixed in the gel by 20% (wt/vol) trichloroacetic acid at 4°C and stained with 0.05% Coomassie Brilliant Blue R250 dissolved in 20% (wt/vol) methanol. Radiolabeled IgG were located by autoradiography on prefogged Kodak X-Omat AR film (Eastman Kodak Co., Rochester, NY) at –80°C (18).

Proteolytic Reaction Mixtures. Macrophage elastase was incubated at 37°C with IgG (1:100, wt/wt) in 18 mM Tris-HCl, pH 8.0, containing 10 mM CaCl₂ and 30 mM NaCl, for 1–20 h. Usually 0.1 µg of macrophage elastase (0.5 U, where 1 U degraded 1 µg of insoluble elastin/h) was incubated with 10 µg of immunoglobulin. Typical reactions were carried out in 15-µl vol with reactant concentrations of 6.67 µg/ml (33.33 U/ml) elastase and 0.67 mg/ml immunoglobulin. When Fc fragments were used as substrates they were incubated at a 1:100 ratio in 0.15 M Tris-acetate buffer, pH 7.4. Reactions were stopped by the addition of EDTA to a final concentration of 30 mM. When proteinase inhibitors were included in the reaction mixtures, they were preincubated with macrophage elastase for 30 min at room temperature. PMSF was dissolved in isopropanol and used at 1 mM. 1,10-Phenanthroline was dissolved in ethanol and used at 1 mM. Inhibition was determined with reference to solvent controls. Ac(Ala)₄CH₂Cl and α_1 -proteinase inhibitors were prepared in Tris-HCl, pH 8.0.

Radiolabeling of IgG. Radioiodination of IgG_{2a} was done with Na¹²⁵I by the chloramine T method (19). IgG₃ was biosynthetically labeled by growing N-S.7 cells for 24 h in methionine-free Dulbecco's modified Eagle's medium containing [³⁵S]methionine (25 µCi/ml). The conditioned medium was adjusted to pH 8.0 with solid Tris base and passed over a protein A-Sepharose column (9 × 0.9 cm). Chromatography was carried out at 30 ml/h at 4°C. IgG₃ was eluted with 0.1 M citrate buffer, pH 3.0 (20). Fractions were collected into sufficient 1 M Tris base to give a final pH of 7.0–7.5. Fractions containing IgG₃ were pooled and concentrated on an XM50 membrane with the buffer changed to 0.05 M Tris-HCl, pH 8.0.

Adsorption of IgG Cleavage Products to Protein A. Intact IgG or IgG-macrophage elastase reaction mixtures were incubated at 0°C in 0.01 M Tris-HCl, 0.015 M CaCl₂, pH 8.0, for 40 min with sufficient excess IgSorb (formalin-fixed *Staphylococcus aureus*, Cowan strain I, containing protein A) to bind twice the amount of IgG present. The protein-IgSorb complexes were collected by centrifugation for 5 min in a Microfuge B (Beckman Instruments, Inc., Palo Alto, CA). The supernatants contained the material lacking an Fc region, and the pellet contained intact IgG

and proteolytic fragments with Fc regions. The pellet was washed with 0.01 M Tris-HCl, pH 8.0. Both supernatant and pellet were then analyzed by SDS-polyacrylamide gel electrophoresis under nonreducing conditions.

Fc Receptor Binding Studies. P388D1 macrophages, maintained in culture by established methods (21), were used to evaluate the ability of IgG_{2a} cleaved by macrophage elastase to compete with intact [¹²⁵I]IgG_{2a} for the specific IgG_{2a} FcR. Detailed methods for the competition experiments are described elsewhere (1, 2).

Fc Fragments. IgG_{2a} (UPC 10) (2 mg) was incubated with papain (1:500 enzyme/substrate, wt/wt) for 12 h at 37°C in 0.02 M Tris-buffered saline, 0.01 M EDTA, pH 8.1. The reaction mixture was then passed over a protein A-Sepharose column at 4°C. Proteolytic fragments without Fc regions were contained in the pH 8.0 eluate. Intact IgG_{2a} and Fc fragments were eluted together at pH 3.0 and then concentrated by ultrafiltration on a YM10 membrane. IgG_{2a} was fully separated from the Fc fragments after size-exclusion chromatography on a Sphergel-TSK 2000-SW column (Altex, Berkeley, CA) in 0.15 M Tris-acetate, pH 7.4, at 1.0 ml/min on a 1084B liquid chromatograph (Hewlett-Packard Co., Palo Alto, CA). Intact IgG_{2a} eluted at 13.5 min and the 58,000-mol wt Fc fragment eluted at 21.6 min. No other peaks were detected at 280 nm. Molecular weights were determined on SDS-polyacrylamide electrophoresis gels under nonreducing conditions.

Results

Proteolysis of IgG Subclasses by Macrophage Elastase. Monoclonal mouse IgG₁, IgG_{2a}, IgG_{2b}, and IgG₃ were used as substrates for purified mouse macrophage elastase. Of these IgG subclasses, only IgG₁ was resistant to degradation (Fig. 1). When analyzed by SDS-polyacrylamide gel electrophoresis under nonreducing conditions (Fig. 1), the proteolysis of IgG_{2a} and IgG₃ resulted in nearly identical major peptides with sizes of 105,000 and 24,000 mol wt (IgG_{2a}) and 113,000 and 23,000 mol wt (IgG₃), as well as

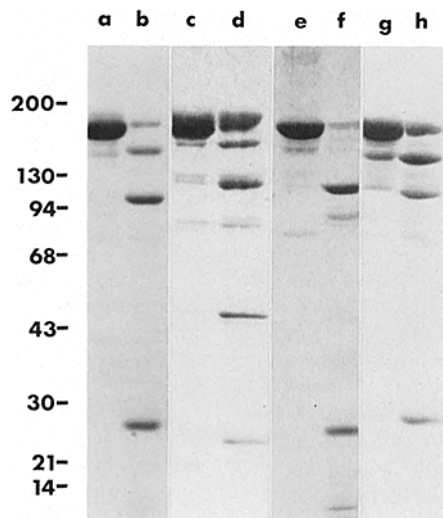


FIG. 1. Limited proteolysis of IgG by macrophage elastase. SDS-polyacrylamide gradient gels with samples applied unreduced. Lanes *a* and *b* show IgG_{2a} (UPC 10); *c* and *d* IgG_{2b} (MOPC 141); *e* and *f* IgG₃ (FLOPC 21); *g* and *h* human IgG₁. Lanes *a*, *c*, *e*, and *g* show 15 h incubation of IgG with macrophage elastase inhibited with 10 mM EDTA. Lanes *b*, *d*, *f*, and *h* show IgG incubated with active macrophage elastase for 15 h. Molecular weight markers ($\times 10^{-3}$) are shown at left. The major cleavage fragments produced under nonreducing conditions from IgG_{2a} (lane *b*) were 144,000, 105,000, and 24,000 mol wt; from IgG_{2b} (lane *d*), 151,000, 116,000, 89,000, 50,000, and 22,000 mol wt; from IgG₃ (lane *f*), 113,000, 96,000, 23,000, and 14,000 mol wt; from human IgG₁ (lane *h*), 137,000, 109,000, and 28,000 mol wt.

a minor cleavage peptide of 144,000 mol wt from IgG_{2a}. The proteolysis of IgG_{2b} was more complicated. Not all forms of monoclonal IgG_{2b} were degraded. There was no detectable degradation of IgG_{2b} secreted by Sp2/HL, whereas IgG_{2b} secreted by 10-2.16, MOPC 195, and MOPC 141 were degraded nearly to completion. The proteolysis of IgG_{2b} resulted in the production of approximately the same size major peptides detected from IgG_{2a} and IgG₃, plus two additional peptides of 89,000 and 50,000 mol wt.

Because it binds to the mouse IgG_{2a} FcR, we also used human IgG₁ as a substrate for macrophage elastase (Fig. 1). The resulting proteolytic peptides were identical to those produced by the proteolysis of IgG_{2a} and IgG₃.

Macrophage elastase also degraded mouse IgM and IgA. However, the cleavage of these immunoglobulins was more limited than the cleavage of IgG. The subclasses of immunoglobulins degraded by macrophage elastase are summarized in Table I.

Time Course of Proteolysis of IgG. To determine the rate at which macrophage elastase degrades immunoglobulins, IgG_{2a}, and a susceptible IgG_{2b} were incubated for times up to 20 h (Fig. 2). SDS-polyacrylamide gel electrophoresis under nonreducing conditions showed that at a 1:100 ratio (enzyme/substrate, wt/wt) the proteolysis of IgG_{2a} was detectable after 1 h of incubation but that the 144,000-mol wt peptide was most prominent and remained prominent throughout 15 h of incubation. Proteolysis of IgG_{2a} did not approach completion until the reaction mixtures had been incubated for at least 20 h. This suggests that the proteolysis of IgG_{2a} proceeded in two stages, first the production of the 144,000-mol wt peptide, then further degradation to the 105,000- and 24,000-mol wt peptides. The same results were found with IgG₃ (not shown). In contrast, the more complex degradation of IgG_{2b} proceeded without the apparent accumulation of a particular cleavage peptide.

TABLE I
Proteolysis of Immunoglobulins by Macrophage Elastase

Species	Ig type	Cell type	Light chain type	Degraded by macrophage elastase*
Mouse	IgG ₁	Sp3/HL	ND‡	No
		MOPC 21	κ	No
	IgG _{2a}	UPC 10	κ	Yes
		RPC 5	κ	Yes
	IgG _{2b}	MOPC 141	κ	Yes
		MOPC 195	κ	Yes
		Sp2/HL	ND	No
		10-2.16	ND	Yes
	IgG ₃	FLOPC 21	κ	Yes
		Y 5606	λ	Yes
		N-S.7	κ	Yes
	IgA	MOPC 315	λ	Slightly
Human	IgG ₁	TEPC 183	κ	Slightly
				Yes

* Degradation was assessed at a 1:100 ratio (enzyme/substrate, wt/wt) and was considered positive if discrete cleavage peptides were detectable after 20 h at 37°C by SDS-polyacrylamide gel electrophoresis. At least two experiments were performed for each Ig type.

‡ Not determined.

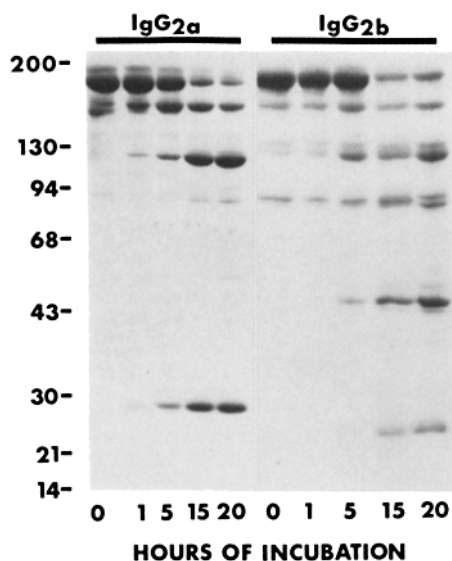


FIG. 2. Time course of the degradation of IgG by macrophage elastase. Reaction mixtures of macrophage elastase with IgG_{2a} (UPC 10) or IgG_{2b} (MOPC 141) were incubated for the times indicated, then analyzed unreduced on SDS-polyacrylamide gradient gels. Molecular weight markers ($\times 10^{-3}$) are shown at left.

Effect of Proteinase Inhibitors on the Degradation of IgG by Macrophage Elastase. To demonstrate that the cleavage of IgG_{2a} is the result of proteolysis by macrophage elastase, we investigated the effect of proteinase inhibitors that distinguish metalloproteinases from serine proteinases. Some of these inhibitors specifically distinguish macrophage elastase activity from granulocyte elastase activity. Fig. 3 demonstrates that, of the inhibitors used, only the metal chelators, 1,10-phenanthroline and EDTA, at 1 mM, inhibited macrophage elastase-mediated proteolysis of IgG. Neither PMSF (1 mM), a general serine proteinase inhibitor, nor Ac(Ala)₄CH₂Cl (0.1 mM), a specific inhibitor of both granulocyte and pancreatic elastase (22), inhibited degradation more than solvent controls (not shown). Organic solvents used to solubilize these inhibitors are slightly inhibitory to macrophage elastase (14). Because the metal chelator 1,10-phenanthroline completely inhibited degradation, the limited proteolysis of immunoglobulins seen in this study could not be attributed to contamination by serine proteinases. The serum inhibitor of serine proteinases, α_1 -proteinase inhibitor, also did not prevent degradation of IgG_{2a} by macrophage elastase, but rather acted as a competitive substrate for macrophage elastase (15), resulting in less than complete degradation of IgG_{2a}.

Characterization of Proteolytic Fragments. To determine which chain of the immunoglobulin is recognized and cleaved by macrophage elastase, we analyzed the proteolytic reaction mixtures under reducing conditions on SDS-polyacrylamide electrophoresis gels. Fig. 4 shows that macrophage elastase-mediated proteolysis of IgG_{2a}, IgG_{2b}, and IgG₃ was restricted to the heavy chain and that IgG₁ was resistant to degradation. Under these conditions the major proteolytic fragments of IgG_{2a} migrated as peptides of 33,000 and 31,000 mol wt, with minor small fragments. These gels also showed the accumulation of a 26,000-mol wt peptide from IgG_{2a} that was approximately the same size as the light chain. The analysis of these reaction mixtures under nonreducing

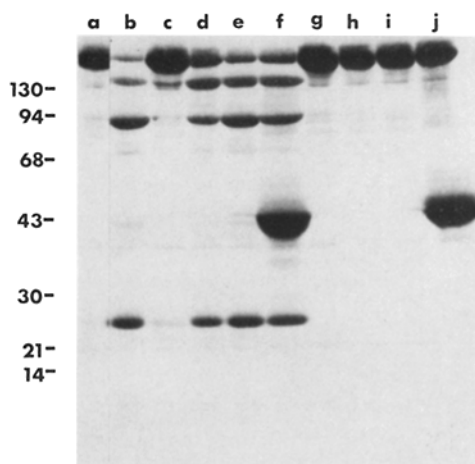


FIG. 3. Effect of proteinase inhibitors on the proteolysis of IgG_{2a} (UPC 10) by macrophage elastase. SDS-polyacrylamide gradient gel. Lane *a*, IgG_{2a} incubated with EDTA-inhibited macrophage elastase at 37°C for 15 h; *b*, IgG_{2a} incubated with active macrophage elastase at 37°C for 20 h. In lanes *c-f*, IgG_{2a} was incubated for 20 h at 37°C with active macrophage elastase plus (*c*) 1 mM 1,10-phenanthroline; *d*, 0.1 mM Ac(Ala)₄CH₂Cl; *e*, 1 mM PMSF; *f*, 1 mg/ml α_1 -proteinase inhibitor. Lanes *g-j* show IgG_{2a} incubated with the same concentration of inhibitors as in *c-f* but without macrophage elastase. Cleavage seen in lanes *d* and *e* was indistinguishable from that seen with elastase in the presence of solvent alone (not shown). Molecular weight markers ($\times 10^{-3}$) are shown at left.

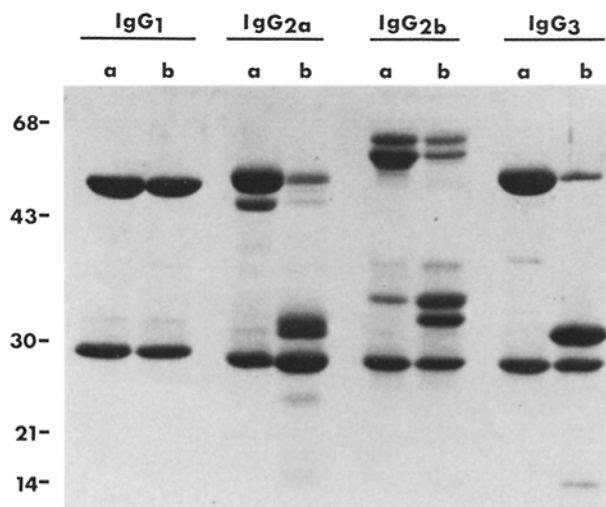


FIG. 4. Limited proteolysis of the heavy chain of IgG by macrophage elastase. Reaction mixtures of macrophage elastase with IgG₁ (MOPC 21), IgG_{2a} (UPC 10), IgG_{2b} (MOPC 141), or IgG₃ (FLOPC 21) were analyzed after reduction (0.5% 2-mercaptoethanol at 100°C for 5 min) on SDS-polyacrylamide gradient gels. Lanes marked *a* contain IgG incubated for 24 h without macrophage elastase. Lanes marked *b* contain IgG incubated for 20 h with macrophage elastase. Molecular weight markers ($\times 10^{-3}$) are shown at left. The cleavage fragments produced under reducing conditions from IgG_{2a} were 33,000, 31,000, 26,000 (co-migrating with and slightly faster than the light chain), 23,000, and 16,000 mol wt; from IgG_{2b}, 35,000 and 33,000 mol wt; from IgG₃, 31,000 and 14,000 mol wt. The 31,000-mol wt peptide produced from IgG₃ frequently resolved as a doublet.

conditions (Fig. 1) showed the production of a 24,000-mol wt fragment. This fragment migrated with or near the light chain in the gel shown in Fig. 4. The degradation of IgG₃ produced peptides of 31,000 and 14,000 mol wt. The 31,000-mol wt peptide frequently resolved as a doublet. The degradation of IgG_{2b} produced peptides of 35,000 and 33,000 mol wt.

Analysis by gel electrophoresis (Fig. 4) did not indicate any loss of the light chain, and susceptibility of IgG to cleavage by macrophage elastase could not be correlated to light chain type (Table I). IgG₁ (MOPC 21) was kappa light chain but was not cleaved, whereas several kappa light chain IgG_{2a} and IgG_{2b} substrates were cleaved. All three forms of IgG₃ that were tested were cleaved; FLOPC 21 and N-S.7 were kappa light chain, and Y5606 was lambda light chain.

Because the heavy chain of IgG is degraded by macrophage elastase, we performed experiments to determine which proteolytic fragment contained the Fc region. To characterize the fragments resulting from macrophage elastase proteolysis of IgG_{2a}, IgG_{2b}, and IgG₃, reaction mixtures were adsorbed with IgSorb, which binds Fc fragments because of the interaction of the C_H3 domain with protein A. The 24,000-mol wt fragment was adsorbed to protein A (Fig. 5), which indicates that this fragment contained the Fc' portion of IgG_{2a}. Because only the heavy chain of IgG_{2a} is degraded by macrophage elastase, coincident with the appearance of a band slightly smaller than the light chain (Fig. 4), this 24,000-mol wt fragment is most likely the C_H2 and C_H3 domain of IgG_{2a}, which is consistent with an Fc'-size fragment. The 33,000- and 31,000-mol wt fragments, which were also visible on reduced gels (Fig. 4), are two possible cleavage sites in the heavy chain. The 105,000-mol wt fragment that was not bound by protein A (Fig. 5) lacks an Fc region and is consistent with an F(ab')₂-size fragment. The 144,000-mol wt intermediate degradation product (Fig. 2)

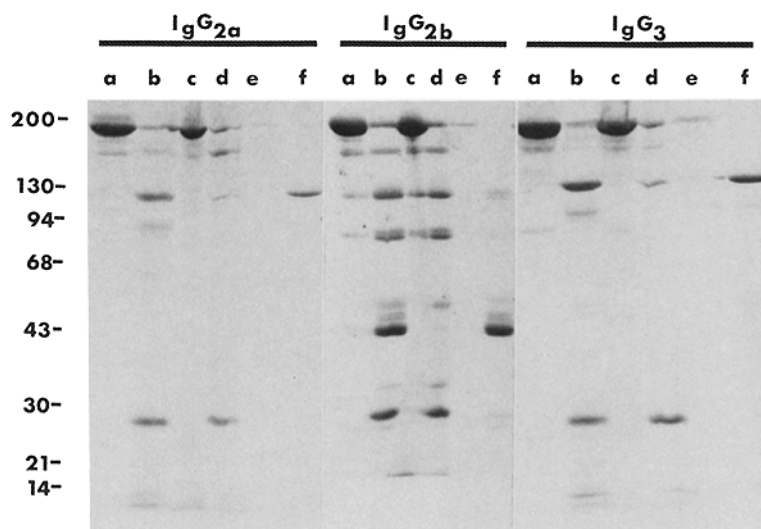


FIG. 5. Protein A absorption of IgG_{2a} (UPC 10), IgG_{2b} (10-2.16), and IgG₃ (FLOPC 21) after degradation by macrophage elastase. Lanes contain (a) intact control IgG; (b) 20 h reaction mixture with macrophage elastase; (c) intact IgG adsorbed to protein A; (d) reaction mixtures of IgG and macrophage elastase adsorbed to protein A (pellet); (e) material from intact IgG not adsorbed to protein A (supernatants); and (f) material from reaction mixtures not adsorbed to protein A. Molecular weight markers ($\times 10^{-3}$) are shown at left.

was also bound by protein A. This is consistent with an elastase cleavage near the hinge region in the C_H2 domain, resulting in the release of a single Fc' fragment and leaving the molecule with one intact heavy chain that could still bind to protein A. The proteolytic fragments of IgG₃ were similar to those of IgG_{2a} with respect to protein A binding (Fig. 5).

All of the proteolytic fragments of IgG_{2b} were absorbed by protein A except the 50,000-mol wt peptide. It is likely that the IgG_{2b} cleavage points are above the hinge region at the end of the C_H1 domain and below the hinge region at the beginning of the C_H2 domain.

Effect of Proteolysis by Macrophage Elastase on the Binding of IgG_{2a} to FcR. We performed an experiment to determine whether the fragments resulting from the limited proteolysis of IgG by macrophage elastase can compete with intact IgG for binding to the FcR. IgG_{2a} was used as a substrate for macrophage elastase because the IgG_{2a} FcR binds monomeric IgG_{2a}. Aliquots of the reaction mixtures shown in Fig. 5 or intact IgG_{2a} were combined with a constant amount of [¹²⁵I]IgG_{2a} and incubated with P388D1 macrophages (Table II). Intact IgG_{2a} inhibited binding of [¹²⁵I]IgG_{2a} by 71%. After 20 h of incubation with macrophage elastase, the degraded IgG_{2a} would no longer efficiently bind to the FcR, as indicated by a reduction to only 27% inhibition of binding of [¹²⁵I]IgG_{2a}. Reaction mixtures that included the metal chelator, 1,10-phenanthroline (1 mM) inhibited the macrophage elastase proteolytic activity, allowing sufficient intact IgG_{2a} remaining in the reaction mixture to inhibit the binding of [¹²⁵I]IgG_{2a} by 62%.

Proteolysis of Immune Complexes by Macrophage Elastase. The preceding experiments described the limited proteolysis of monomeric IgG by macrophage elastase. However, the conformation of the IgG molecule is altered in an immune complex; new proteolytic sites may be exposed while previously exposed ones are protected. Because of the conformational changes in an immunoglobulin involved in an immune complex, a series of experiments were performed to determine the ability of macrophage elastase to cleave IgG₃ bound to SRBC. The IgG₃ anti-SRBC that was biosynthetically labeled with [³⁵S]methionine was used to opsonize SRBC. The reaction mixtures of these immune complexes with macrophage elastase were analyzed by autoradiography of SDS-polyacrylamide gels that were run under reducing conditions. A densitometric scan of the autoradiograph showed that macrophage elastase degraded IgG₃ bound

TABLE II
Competition of Macrophage Elastase (ME)-degraded IgG_{2a} with [¹²⁵I]IgG_{2a} for Binding to P388D1 IgG_{2a} FcR

Competing reaction mixture	Incubation time	Inhibition of [¹²⁵ I]-IgG _{2a} binding
	<i>h</i>	%
None		0
Intact IgG _{2a}	20	71
ME-IgG _{2a}	15	40
ME-IgG _{2a}	20	27
ME-IgG _{2a} + PMSF (1 mM)	20	30
ME-IgG _{2a} + 1,10-phenanthroline (1 mM)	20	62

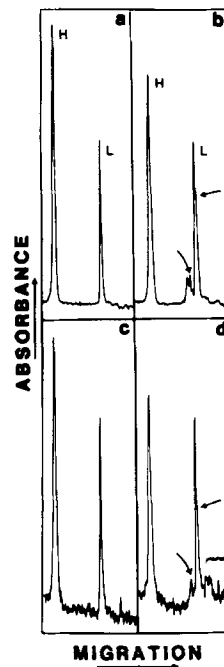


FIG. 6. Densitometric scan of an autoradiograph of SDS-polyacrylamide gradient gels of reduced [35 S]methionine-labeled mouse IgG₃ anti-SRBC (N-S.7). Panel *a* shows intact soluble IgG₃ anti-SRBC, *b* shows macrophage elastase-degraded soluble IgG₃ anti-SRBC, *c* shows intact IgG₃ anti-SRBC that was bound to SRBC, and *d* shows IgG₃ anti-SRBC degraded by macrophage elastase while bound to SRBC. The arrows in panels *b* and *d* indicate the cleavage fragments common to both soluble and immune-complexed IgG₃-macrophage elastase reaction mixtures. The bracket in panel *d* indicates cleavage fragments characteristic of immune-complexed IgG₃-macrophage elastase reaction mixtures. The relative positions of the heavy (H) and light (L) chains are indicated in panels *a* and *b*.

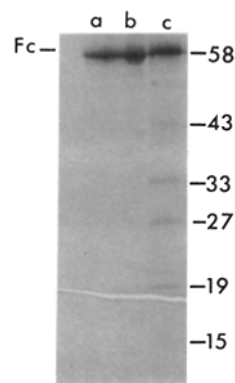


FIG. 7. Degradation of IgG_{2a} (UPC 10) Fc fragments by macrophage elastase. Samples were applied unreduced to SDS-polyacrylamide gradient gels. (*a*) Unincubated Fc fragment; (*b*) Fc fragment incubated for 5 h at 37°C without macrophage elastase; (*c*) Fc fragment incubated for 1 h at 37°C with macrophage elastase. Molecular weights of the degraded Fc fragments ($\times 10^{-3}$) are shown at right.

in an immune complex (Fig. 6). Although the cleavage by macrophage elastase of IgG₃ in immune complexes differed from the cleavage of monomeric IgG₃, there remain some important similarities. Whereas the cleavage of monomeric IgG₃ gave two cleavage products, 32,000 and 31,000 mol wt, of the heavy chain, Fig. 6 indicates that the proteolysis of immune-complexed IgG₃ was still limited to the heavy chain but that the 32,000-mol wt heavy chain fragment was not a degradation product. With immune-complexed IgG₃ there was also an increase in the production of fragments smaller than the light chain. One result of the removal of the Fc portion of IgG₃ by macrophage elastase would be that the IgG₃-SRBC immune complex could not be bound by the FcR and therefore would not be phagocytized.

Proteolysis of the Fc Fragments by Macrophage Elastase. Fc fragments generated in tissues by proteinases such as granulocyte elastase are conformationally different from the Fc region of the intact immunoglobulin. We investigated the possibility that an Fc fragment produced by another proteinase would present additional sites for proteolysis by macrophage elastase. A 58,000-mol wt papain-generated Fc fragment of IgG_{2a}, which is similar to an Fc fragment produced by granulocyte elastase, was incubated for up to 5 h with macrophage elastase. Fig. 7 shows that degradation of this fragment was detected after only 1 h of incubation at pH 7.4. The fragments produced had apparent molecular weights from 43,000 to 15,000 mol wt on nonreducing SDS-polyacrylamide gels.

Discussion

We have shown that mouse macrophage elastase selectively cleaves mouse IgG_{2a}, IgG₃, and human IgG₁ into F(ab')₂-like and Fc'-like subfragments. These peptides are similar to the proteolytic fragments produced by pepsin rather than those produced by papain. Not all of the monoclonal IgG_{2b} preparations that we investigated were degraded by macrophage elastase, and the IgG_{2b} forms that were susceptible were degraded into more peptide fragments than were IgG_{2a} and IgG₃.

The susceptibility of immunoglobulins to cleavage was independent of the type of light chain because immunoglobulins were degraded whether they contained kappa or lambda light chain. There was no indication of the cleavage of light chain in any of the experiments performed. The susceptibility of IgG to proteolysis by macrophage elastase did vary with the conformational state of the IgG molecule. The proteolysis of IgG₃ in immune complexes differed from the proteolysis of monomeric IgG₃. The apparent two-stage degradation of monomeric IgG_{2a} and IgG₃ suggests that the conformation of the first proteolysis product, 144,000 mol wt, where one heavy chain had been cleaved, was less favorable for degradation than was the intact molecule.

The time course of proteolysis (Fig. 2) indicates that immunoglobulins are less favored substrates for macrophage elastase than is α_1 -proteinase inhibitor. When these two proteins were used as substrates under identical conditions (1:100 enzyme/substrate, wt/wt), all of the native α_1 -proteinase inhibitor was degraded in 4 h (15), whereas the total degradation of native IgG (this study) required at least 20 h. α_1 -Proteinase inhibitor was degraded at a rate of $9.48 \text{ mol h}^{-1} (\text{mol elastase})^{-1}$, whereas IgG was degraded at $0.67 \text{ mol h}^{-1} (\text{mol elastase})^{-1}$, a 14-fold lower rate. Also, when α_1 -proteinase inhibitor was used in the inhibitor experiments with macrophage elastase (Fig. 3), it was cleaved preferentially.

The degradation of IgG by macrophage elastase could possibly interfere with FcR

functions (23). Of the susceptible IgG subclasses, IgG_{2a} and IgG₃ have different receptors, whereas IgG₁ and IgG_{2b} share an FcR distinct from the IgG_{2a} FcR and the IgG₃ FcR (3). Whether the fact that IgG₁ and some forms of IgG_{2b} were poorly degraded by macrophage elastase is coincidental or indicative of a regulatory scheme involving proteolysis of IgG and the interaction with the FcR is not known. Guinea pig peritoneal macrophages rapidly degrade soluble immune complexes once the complexes are bound to the FcR (24). The amount of elastase used in the present study (0.5 U) was equivalent to the active elastase present near the surface of as few as 5×10^4 inflammatory macrophages (25).

The data presented here show that degradation by macrophage elastase prevents binding of monomeric IgG to the FcR and suggest that phagocytosis of immune-complexed IgG is also impaired because of degradation by macrophage elastase. Because the integrity of the Fc region is required for antigen-antibody complex-mediated suppression (26), macrophage elastase may also interfere with suppression.

The nature of the cleavage of IgG by mouse macrophage elastase differs from the cleavage of IgG mediated by granulocyte elastase. Human granulocyte elastase, a serine proteinase, readily produces papain-like cleavages in human IgG₁ and IgG₃ and to a lesser extent IgG₂ and IgG₄ (27, 28). The result is the production of Fab and Fc fragments rather than the F(ab')₂-like and Fc'-like subfragments produced by mouse macrophage elastase, a metalloproteinase.

More intriguing is the possible role that degradation of IgG and Fc fragments by macrophage elastase might have in lymphocyte activation and spleen cell mitogenesis. Fc fragments generated by papain, like those produced by granulocyte elastase, can induce mouse B cells to proliferate (8). These fragments also cause B cells to differentiate into polyclonal antibody-secreting cells (8, 9). Macrophages are required for B cell activation (29) because they secrete proteinase into the culture supernatant that converts the papain-produced Fc fragment into a smaller peptide with mitogenic activity. The active fragment resulting from the macrophage-mediated degradation of Fc fragments elutes from a gel filtration column as a broad peak with an average of 14,000 mol wt (30) and more recently has been shown to be derived from the C_H3 domain of human IgG₁ (31). Macrophage elastase releases a fragment containing the C_H3 domain from intact mouse IgG as well as from human IgG₁. Although the 24,000-mol wt Fc fragment was the major product of elastase degradation of intact IgG by macrophage elastase in this study, minor fragments as small as 14,000 mol wt were also produced (Figs. 4 and 5). It is therefore conceivable that in vivo macrophage elastase may participate in the production of mitogenic Fc subfragments from intact IgG. Thus, macrophage elastase may be a key proteolytic regulator of lymphocyte mitogen production.

The data reported here demonstrate that macrophage elastase degrades selected IgG subclasses in a manner qualitatively different from that of proteinases from other inflammatory cells. Because macrophage elastase is a metalloproteinase, it is not subject to inhibition by α_1 -proteinase inhibitor, an inhibitor of serine proteinases. Thus, degradation of immunoglobulins by macrophage elastase may continue in situations in which granulocyte elastase, plasmin, or other serine proteinases are inhibited, resulting in a change in the type of IgG turnover.

In this report we have established the potential for macrophage elastase to be a regulator of some aspects of immunoglobulin-associated cellular events. The biologic

role of the proteolytic fragments of IgG by this macrophage proteinase will require functional studies.

Summary

Mouse macrophage elastase, a metalloproteinase secreted by inflammatory macrophages, catalyzed the limited proteolysis of selected subclasses of mouse immunoglobulins, including monomeric IgG_{2a}, IgG₃, and some forms of IgG_{2b}. Mouse IgG₁ was resistant to elastase degradation; however, human IgG₁ was degraded. IgG₃ in immune complexes was cleaved in a manner similar to that of monomeric IgG₃. Degradation by macrophage elastase was limited to the heavy chain, resulting in products that did not compete for binding to the macrophage Fc receptor. Macrophage elastase usually produced a pepsin-like rather than a papain-like pattern of proteolysis, resulting in the release of F(ab')₂ and Fc' subfragments. This degradation of IgG differed from the papain-like cleavage of IgG by granulocyte elastase. Macrophage elastase degraded papain-generated Fc fragments of IgG_{2a} into multiple fragments. Therefore, macrophage elastase at concentrations found in culture medium has the potential to regulate some aspects of cellular events associated with immunoglobulins.

Received for publication 7 December 1982.

References

1. Unkeless, J. C., and H. N. Eisen. 1975. Binding of monomeric immunoglobulins to Fc receptors of mouse macrophages. *J. Exp. Med.* **142**:1520.
2. Unkeless, J. C. 1977. The presence of two Fc receptors on mouse macrophages: evidence from a variant cell line and differential trypsin sensitivity. *J. Exp. Med.* **145**:931.
3. Diamond, B., and M. D. Scharff. 1980. IgG1 and IgG2b share the Fc receptor on mouse macrophages. *J. Immunol.* **125**:631.
4. Diamond, B., and D. E. Yelton. 1981. A new Fc receptor on mouse macrophages binding IgG₃. *J. Exp. Med.* **153**:514.
5. Ralph P., I. Nakoinz, B. Diamond, and D. Yelton. 1980. All classes of murine IgG antibody mediate macrophage phagocytosis and lysis of erythrocytes. *J. Immunol.* **125**:1885.
6. Morgan, E. L., S. M. Walker, M. L. Thoman, and W. O. Weigle. 1980. Regulation of the immune response. I. The potentiation of in vivo and in vitro immune responses by Fc fragments. *J. Exp. Med.* **152**:113.
7. Passwell, J., F. S. Rosen, and E. Merler. 1980. The effect of Fc fragments of IgG on human mononuclear cell responses. *Cell. Immunol.* **52**:395.
8. Berman, M. A., and W. O. Weigle. 1977. B-lymphocyte activation by the Fc region of IgG. *J. Exp. Med.* **146**:241.
9. Berman, M. A., H. L. Spiegelberg, and W. O. Weigle. 1979. Lymphocyte stimulation with Fc fragments. I. Class, subclass, and domain of active fragments. *J. Immunol.* **122**:89.
10. Morgan, E. L., M. L. Thoman, and W. O. Weigle. 1981. Enhancement of T lymphocyte functions by Fc fragments of immunoglobulins. I. Augmentation of allogenic mixed lymphocyte culture reactions requires I-A or I-B-subregion differences between effector and stimulator cell populations. *J. Exp. Med.* **153**:1161.
11. Morgan, E. L., and W. O. Weigle. 1981. Regulation of the immune response. III. The role of macrophages in the potentiation of the immune response by Fc fragments. *J. Immunol.* **126**:1302.
12. Birshtein, B. K., R. Campbell, and B. Diamond. 1982. Effects of immunoglobulin structure

- on Fc receptor binding: a mouse myeloma variant immunoglobulin with a $\gamma 2b$ - $\gamma 2a$ hybrid heavy chain having a complete $\gamma 2a$ Fc region fails to bind to $\gamma 2a$ Fc receptors on mouse macrophages. *J. Immunol.* **129**:610.
13. Werb, Z., and S. Gordon. 1975. Elastase secretion by stimulated macrophages. Characterization and regulation. *J. Exp. Med.* **142**:361.
 14. Banda, M. J., and Z. Werb. 1981. Mouse macrophage elastase. Purification and characterization as a metalloproteinase. *Biochem. J.* **193**:589.
 15. Banda, M. J., E. J. Clark, and Z. Werb. 1980. Limited proteolysis by macrophage elastase inactivates human α_1 -proteinase inhibitor. *J. Exp. Med.* **152**:1563.
 16. Oi, V. T., P. P. Jones, J. W. Goding, L. A. Herzenberg, and L. A. Herzenberg. 1978. Properties of monoclonal antibodies to mouse Ig allotypes, H-2 and Ia antigens. In *Current Topics in Microbiology and Immunology: Lymphocyte Hybridomas*. F. Melchers, M. Potter, and N. Warner, editors. Springer-Verlag, New York. **81**:115.
 17. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)*. **227**:680.
 18. Laskey, R. A., and A. D. Mills. 1975. Quantitative film detection of ^3H and ^{14}C in polyacrylamide gels by fluorography. *Eur. J. Biochem.* **56**:335.
 19. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131 labeled human growth hormone of high specific activity. *Nature (Lond.)*. **194**:495.
 20. Ey, P. L., S. J. Prowse, and C. R. Jenkin. 1978. Isolation of pure IgG₁, IgG_{2a} and IgG_{2b} immunoglobulins from mouse serum using protein A-Sepharose. *Immunochemistry*. **15**:429.
 21. Werb, Z., R. Foley, and A. Munck. 1978. Glucocorticoid receptors and glucocorticoid-sensitive secretion of neutral proteinases in a macrophage line. *J. Immunol.* **121**:115.
 22. Powers, J. C., and P. M. Tuhy. 1973. Active-site specific inhibitors of elastase. *Biochemistry*. **12**:4767.
 23. Nitta, T., and T. Suzuki. 1982. Fc γ_{2b} receptor-mediated prostaglandin synthesis by a murine macrophage cell line (P388D₁). *J. Immunol.* **128**:2527.
 24. Daha, M. R., and L. A. van Es. 1982. The role of cellular Fc and C3 receptors on the complement-dependent degradation of stable soluble immunoglobulin aggregates by normal and trypsin-treated peritoneal macrophages. *Immunology*. **47**:203.
 25. Werb, Z., M. J. Banda, and P. A. Jones. 1980. Degradation of connective tissue matrices by macrophages. I. Proteolysis of elastin, glycoproteins, and collagen by proteinases isolated from macrophages. *J. Exp. Med.* **152**:1340.
 26. Morgan, E. L., and C. H. Tempelis. 1978. The requirement for the Fc portion of antibody in antigen-antibody complex-mediated suppression. *J. Immunol.* **120**:1669.
 27. Solomon, A., M. Gramse, and K. Havemann. 1978. Proteolytic cleavage of human IgG molecules by neutral proteases of polymorphonuclear leukocytes. *Eur. J. Immunol.* **8**:782.
 28. Baici, A., M. Knopfel, K. Fehr, F. Skvaril, and A. Boni. 1980. Kinetics of the different susceptibilities of the four human immunoglobulin G subclasses to proteolysis by human lysosomal elastase. *Scand. J. Immunol.* **12**:41.
 29. Morgan, E. L., and W. O. Weigle. 1979. The requirement for adherent cells in the Fc fragment-induced proliferative response of murine spleen cells. *J. Exp. Med.* **150**:256.
 30. Morgan, E. L., and W. O. Weigle. 1980. Regulation of Fc fragment-induced murine spleen cell proliferation. *J. Exp. Med.* **151**:1.
 31. Morgan, E. L., T. E. Hugli, and W. O. Weigle. 1982. Isolation and identification of a biologically active peptide derived from the CH3 domain of human IgG₁. *Proc. Natl. Acad. Sci. USA*. **79**:5388.